

# The *LSP1- $\alpha$* gene of *Drosophila melanogaster* exhibits dosage compensation when it is relocated to a different site on the X chromosome

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**The *LSP1- $\alpha$*  gene of *Drosophila melanogaster* is located on the X chromosome at 11B yet is not dosage compensated. In order to determine if this gene is inherently incapable of dosage compensation or if it does not compensate because the appropriate regulatory *cis*-acting sequences are absent from its chromosomal domain, we have undertaken to relocate it to ectopic sites on the X chromosome. To differentiate between the transcripts produced by the transduced gene and those produced by the indigenous gene, we inserted a 500-bp sequence of mouse DNA into the *LSP1- $\alpha$*  clone prior to using it for transformation. Our results show that the *LSP1- $\alpha$*  gene exhibits equivalent levels of transcripts in the two sexes when it is relocated to either an autosomal site or to an ectopic site on the X chromosome. We conclude that the *LSP1- $\alpha$*  gene is capable of dosage compensation.**

**Key words:** dosage compensation/*LSP1- $\alpha$* /gene relocation/transformation

## Introduction

Males and females of *Drosophila melanogaster* compensate for the difference in dosage of their X-linked genes by enhancing the transcriptional activity of most of these genes in males (see Jaffe and Laird, 1986, and Lucchesi and Manning, 1987, for recent reviews of the phenomenon of dosage compensation). The ability to compensate, i.e. to hyperactivate in a male, appears to be conferred by closely linked *cis*-acting sequences. Evidence supporting this contention derives from the relocation of cloned X-linked genes to an autosomal site by P-element-mediated germ line transformation (Hazelrigg *et al.*, 1984; Levis *et al.*, 1985; Pirrotta *et al.*, 1985; Krumm *et al.*, 1985; McNabb and Beckendorf, 1986). These transduced genes continue to produce more product in males than in females and appear, therefore, to be able to respond to the regulatory mechanism of dosage compensation from their ectopic locations. In complementary experiments, cloned autosomal genes relocated to the X chromosome are often found to exhibit dosage compensation, suggesting that they have come under the influence of X-linked regulatory elements (Scholnick *et*

*al.*, 1983; Spradling and Rubin, 1983; Marsh *et al.*, 1985).

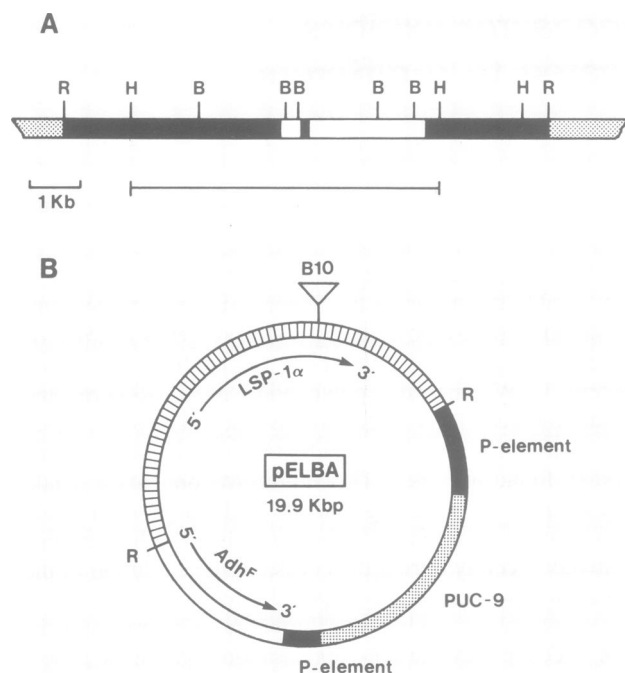
A notable exception to the general rule of dosage compensation of wild-type alleles of X-linked genes is *LSP1- $\alpha$* , the gene which encodes the  $\alpha$ -subunit of the larval serum protein 1. Whether the polypeptide itself (Roberts and Evans-Roberts, 1979) or its RNA (Brock and Roberts, 1982) is measured, female larvae exhibit twice the amount of gene product found in males. This failure to compensate could be explained by assuming that the  $\alpha$ -chain gene has been translocated from an autosome to the X chromosome relatively recently during the course of the evolution of the genus and has not yet developed the means to respond to the compensatory mechanism. Furthermore, there may exist little selective pressure for compensation because the gene's product is interchangeable with that of two autosomal genes encoding very similar larval serum protein 1 subunits, *LSP1- $\beta$*  and *LSP1- $\gamma$*  (Roberts and Evans-Roberts, 1979). During the course of a study of the role of hypersensitive sites on the regulation of LSP genes, Jowett (1985) stated that, in four instances of P-element-mediated transposition of a cloned *LSP1- $\alpha$*  gene to ectopic sites including one on the X chromosome, no dosage compensation was shown by any of the insertions.

The absence of dosage compensation of a wild-type X-linked gene could be due to an inherent property of the gene itself. The kinetics of polymerase binding and of initiation of transcription may be such that the promoter region of a particular gene is rapidly saturated upon activation. The transcription of such a gene would not be amenable to enhancement and the level of product would be dependent on gene dosage. In order to determine if *LSP1- $\alpha$*  is inherently incapable of dosage compensation or if it does not compensate because the appropriate regulatory *cis*-acting sequences are absent from its chromosomal domain, we have undertaken to relocate this gene to ectopic sites on the X chromosome. To differentiate between the transcripts produced by the transduced gene and those produced by the indigenous gene, we inserted a 500 bp sequence of mouse DNA into the *LSP1- $\alpha$*  clone prior to using it for transformation. This hybrid gene produced a longer transcript, easily separable from the normal transcript by electrophoresis. Our results show that the steady-state levels of transcripts of the *LSP1- $\alpha$*  gene relocated to ectopic sites on the X chromosome are equivalent in males (one dose) and females (two doses) and that this gene is, therefore, capable of dosage compensation.

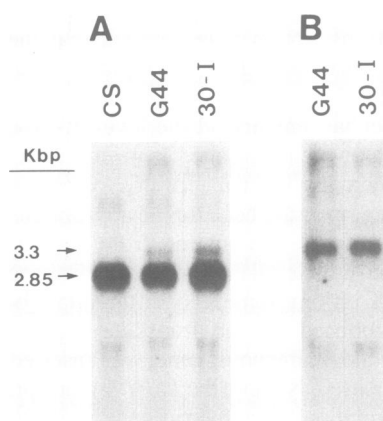
## Results

### **Construction of the transformation vector and recovery of transformants**

A 500 bp *Bam*HI fragment of coding sequence from the mouse L1 repeat was inserted into one of the two 3'-most *Bam*HI sites of the *LSP1- $\alpha$*  gene (Figure 1A). This hybrid gene was introduced as a 9.9 kb *Eco*RI insert into the

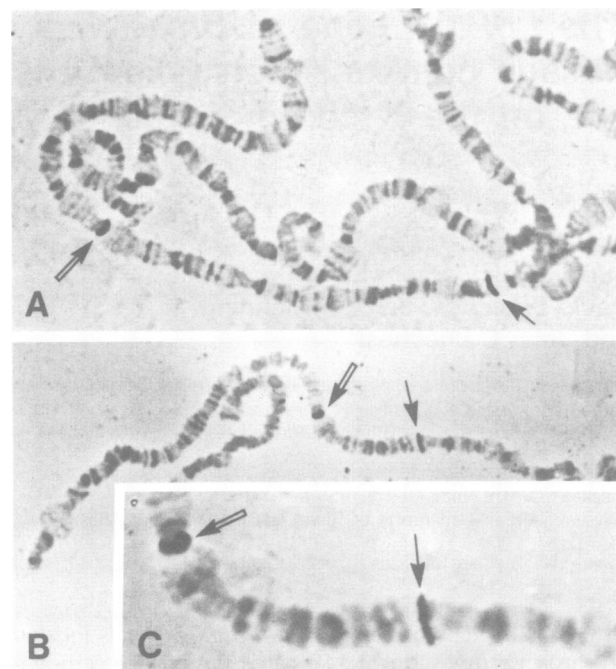


**Fig. 1.** (A) Schematic diagram of the *LSP1-α* clone showing the restriction sites of interest (R, *EcoRI*; H, *HindIII*; B, *BamHI*). For a more complete restriction map, see Brock and Roberts (1983). The solid line represents *Drosophila* genomic DNA; the open boxes are the coding regions of *LSP1-α*; the stippled region is vector DNA. For construction of the pELBA transformation vector, the B10 mouse DNA fragment was inserted into one of the two *HindIII* sites indicated by asterisks. The *HindIII* fragment used as a probe is indicated by the line underneath the *LSP1-α* gene. (B) Structure of the pELBA transformation vector.

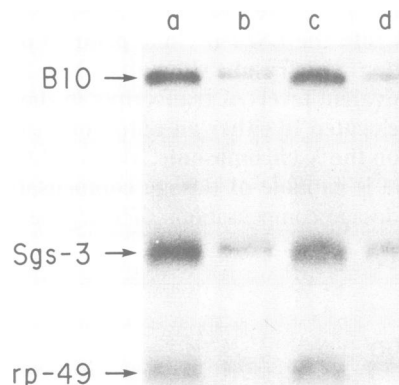


**Fig. 2.** Northern blot analysis of total RNA derived from third instar larvae from a wild-type (CS) strain and from two autosomal transformant lines. (A) The blot was probed with the <sup>32</sup>P-labeled *HindIII* fragment containing *LSP1-α* coding sequences. Note the presence of a larger RNA species in the transformants. (B) The same blot following removal of the *HindIII* probe and hybridization to a <sup>32</sup>P-labeled B10 probe.

pPA-1 transformation vector which contains P-element sequences required for transposition and an *Adh*<sup>+</sup> gene for positive selection of transformants. The new construct, designated pELBA, is diagrammed in Figure 1B. Germline transformations were performed, following the methods and procedures of Rubin and Spradling (1982, 1983) and Karess



**Fig. 3.** Examples of *in situ* hybridization of a biotinylated *HindIII* probe to larval salivary gland polytene chromosomes. The double arrows indicate the site of the indigenous *LSP1-α* gene at 11A and the single arrows indicate the site of insertion of *LSP1-αB10* at 19F in transformant line G44 (A) and at 13DE in transformant line F53 (B). (C) Enlargement of the area of hybridization in line F53.



**Fig. 4.** Determination of the relative steady-state level of *LSP1-αB10* A<sup>+</sup> RNA, *Sgs-3* A<sup>+</sup> RNA and *rp49* A<sup>+</sup> RNA in adult males and females of transformant line F53. Hybridization of the Northern blot was with <sup>32</sup>P-labeled B10 DNA, *Sgs-3* DNA and *rp49* DNA. Lane a, 1.7 μg male RNA; lane b, 0.85 μg male RNA; lane c, 1.74 μg female RNA; lane d, 0.87 μg female RNA. The areas of the blot containing the hybridizing bands were excised and the radioactivity determined. The results are shown in Table I.

and Rubin (1984), by injecting the pELBA vector and the plasmid helper pπ25.7wc into preblastoderm embryos. Transformants were selected among the progeny of G1 individuals by positive selection on ethanol. A total of 1459 embryos were injected yielding 480 adults (33% survival) which produced 21 independent transformation events (4.8%). For those transformants that were homozygous and fertile, permanent lines (a total of eight) were established by repeated inbreeding and selection on ethanol.

### Molecular and cytological characterization of transformant lines

The *LSP1-α* gene is normally active during the third larval instar, leading to a maximum level of steady-state RNA a few hours before pupariation; this level declines slightly as the larvae pupariate (Powell *et al.*, 1984). During the period of peak RNA synthesis, the *LSP1-α* transcript can be readily detected by dot-blot hybridization in total nucleic acid preparations from dissected larval fat bodies (Brock and Roberts, 1982). To determine if the transduced *LSP1-αB10* hybrid gene in transformant lines produced transcripts which could be differentiated from that of the indigenous gene and which were of sufficient stability and abundance to be quantified, total RNA was extracted from late third instar larvae and size fractionated by electrophoresis. Northern blots of this RNA, probed with the 6.0 kb *HindIII* fragment

shown in Figure 1A, revealed a major band of hybridization at 2.85 kb, corresponding to the native *LSP1-α* RNA (Smith *et al.*, 1981), and a second less intense band at 3.3 kb (Figure 2). Since the latter band is seen only in RNA from transformed individuals and is the sole hybridization product observed in Northern blots probed with B10 DNA, it is taken to be the RNA transcript of the transposed *LSP1-αB10* hybrid gene.

The location of the transduced *LSP1-αB10* hybrid genes was determined by *in situ* hybridization of a biotinylated probe consisting of the *HindIII* fragment shown in Figure 1A. All transformant lines exhibited hybridization at 11B on the salivary gland chromosome map, identifying the locus of the indigenous gene (Brock and Roberts, 1983). Two lines had an additional X-linked site (Figure 3) and the remaining six lines an additional autosomal site. One line among the latter was not used in this study because it included some individuals whose glands were apparently mosaic for an additional insertion, a phenomenon previously reported by Krumm *et al.* (1985). The cytological location of the single ectopic sites present in the seven lines studied is provided in Table II. The intensity of the hybridization signal at these ectopic sites and at the normal locus of the *LSP1-α* gene were comparable, indicating that all insertions contained a single copy of the transduced gene. This was consistent with the results of Southern blot analysis of genomic DNA from transformed lines (data not shown).

**Table I.** Analysis of steady-state levels of *LSP1-αB10* transcripts in males and females of transformant line F53

RNA (μg)	c.p.m. hybridized <sup>a</sup> — male			Ratio in males					
	B10	Sgs-3	rp49	B10	B10	Sgs-3			
				Sgs-3	rp49	rp49			
1.70	7189	9574	5662	0.75	1.27	1.70			
0.85	3061	4316	2548	0.71	1.20	1.70			
	c.p.m. hybridized— female			Ratio in female			Male/female		
	B10	Sgs-3	rp49	B10	B10	Sgs-3	B10	B10	Sgs-3
				Sgs-3	rp49	rp49	Sgs-3	rp49	rp49
1.74	6997	9009	6055	0.77	1.16	1.49	0.97	1.09	1.14
0.87	3011	4955	3057	0.61	0.99	1.62	1.16	1.21	1.05

<sup>a</sup>Respective areas of the Northern blot of transformant line F53 showing hybridization in Figure 4 were excised and radioactivity determined. Nylon membrane of an equivalent area was excised where no hybridization was observed and counted to determine background radioactivity. <sup>32</sup>P-labeled probes used were: B10, 500 bp *Bam*HI L1 B10 mouse fragment; Sgs-3, 5.7 kb *Eco*RI fragment from cDM2008; rp49, 639 bp *Eco*RI–*Hind*III fragment from pGRP49.

### Analysis of the expression of transduced genes in transformant lines

In order to determine if the *LSP1-αB10* gene is hyperactive in males (i.e. is subjected to dosage compensation) when it is inserted on the X chromosome, the steady-state level of its transcript was measured in male and female larvae from all of the transformant lines. An example of the approach used for analysis of the *LSP1-αB10* transcript in the two sexes is shown for transformant line F53 (Figure 4, Table I). Following hybridization of Northern blots with a radio-labeled B10 probe, the hybridizing bands corresponding to the *LSP1-αB10* transcript were excised and the radioactivity measured. A direct comparison of the radioactivity in the

**Table II.** Relative steady-state levels of *LSP1-αB10* transcripts in males and females

<i>LSP1-αB10</i> insertion	Cytological position	RNA preparation	c.p.m. hybridized <sup>a</sup> male/female		
			<i>LSP1-αB10</i> Sgs-3	<i>LSP1-αB10</i> rp49	Sgs-3 rp49
F53	13DE (X)	1	1.06 ± 0.12	0.93 ± 0.20	1.08 ± 0.06
		2	0.88 ± 0.18	0.80 ± 0.17	1.00 ± 0.12
G44	19F (X)	1	0.80 ± 0.12	0.83 ± 0.13	1.07 ± 0.25
		2	0.78 ± 0.14	0.87 ± 0.12	1.14 ± 0.23
V24	53A (2R)	1	1.09 ± 0.24	1.00 ± 0.01	1.05 ± 0.14
		2	1.06 ± 0.12	1.24 ± 0.06	1.18 ± 0.12
B26	59A (2R)	1	1.10 ± 0.24	1.26 ± 0.07	1.22 ± 0.25
		2	1.13 ± 0.23	1.18 ± 0.16	1.05 ± 0.25
D21	82B (3R)	1	0.83 ± 0.05	1.06 ± 0.21	1.28 ± 0.25
		2	1.07 ± 0.09	1.07 ± 0.19	0.89 ± 0.01
S13	85CD (3R)	1	0.73 ± 0.10	0.95 ± 0.12	1.32 ± 0.13
		2	0.75 ± 0.13	0.74 ± 0.11	0.98 ± 0.10
B1	98C (3R)	1	0.77 ± 0.07	1.00 ± 0.06	1.28 ± 0.05
		2	0.78 ± 0.11	0.95 ± 0.08	1.21 ± 0.19

<sup>a</sup>Presented in the table are the means of the male-to-female ratios, obtained as described in Table I, and their standard deviations. The number of independent pairs of male and female RNA samples measured for each RNA preparation was three. The analysis presented in Table I represents one independent pair.

bands showed the level of the *LSP1- $\alpha$ B10* transcripts in the male and female larvae to be equivalent at both RNA concentrations tested. However, since some inaccuracy in the amounts of mRNA (i.e. the actual fraction of mRNA in the A + RNA preparation) loaded onto the Northern blot is possible, an internal control to allow for correction of such differences was provided by quantification of the steady-state level of the RNA encoded by the autosomal salivary gland secretion polypeptide gene *Sgs-3* (Korge, 1975; Beckendorf and Kafatos, 1976; Muskavitch and Hogness, 1982) and the ribosomal protein gene *rp49* (O'Connell and Rosbash, 1984). As shown in Table I, the ratio of the steady-state levels of the *LSP1- $\alpha$ B10* transcripts and the autosomal gene transcripts is essentially identical, again indicating that the level of the *LSP1- $\alpha$ B10* transcripts in the two sexes is equivalent. An additional parameter to assess the accuracy of this method of quantification is provided by the measurement of the autosomal gene transcripts, since the male-to-female ratio of *Sgs-3*/*rp49* transcripts should be  $\sim 1.0$  in all transformant lines.

The results of this analysis, presented in Figure 4 and Tables I and II, indicate that in four out of five cases where the *LSP1- $\alpha$ B10* gene is autosomal, there is no difference in the steady-state level of the *LSP1- $\alpha$ B10* transcript between males and females. Although in one of these four transformant lines (B1) the *LSP1- $\alpha$ B10*/*Sgs-3* ratio is significantly  $< 1.0$  (0.78) while the *LSP1- $\alpha$ B10*/*rp49* ratio is  $\sim 1.0$ , it should be noted that in both RNA samples the *Sgs-3*/*rp49* ratio is  $> 1.0$ . This indicates that the *Sgs-3* transcript is more abundant in these RNA samples than the *rp49* transcript, an imbalance which would result in an *LSP1- $\alpha$ B10*/*Sgs-3* ratio of  $< 1.0$ . In one transformant line with an autosomal insert (S13), females clearly had higher levels of gene

product than males. In comparison, one transformant line (F53) with an X-linked transduced gene exhibits equal levels of transcripts in males and females, while the second X-linked transformant line (G44) shows that females have  $\sim 20$ – $25\%$  higher levels of transcripts than males. Since in these two lines males carry a single dose and females carry two doses of the transduced gene, it is legitimate to conclude that the single dose in males is compensated.

To rule out the possibility that the hyperactivation of the single dose of the X-linked *LSP1- $\alpha$ B10* is due, for some reason, to its unpaired condition in males, homozygous females were compared with females bearing a single dose of the transduced gene. The results, presented in Table III, show an  $\sim 50\%$  reduction in gene product in heterozygous females and are consistent with the expected dosage response of a single X-linked gene within a female.

## Discussion

The fundamental aspect of the mechanism of dosage compensation is the ability of most X-linked genes to achieve equal levels of products in males and females by enhancing the transcriptional activity of the single dose in males. It is not sufficient for one X-linked gene or a small cluster of genes to be present in single dose in order to be dosage compensated. There must exist a substantial deviation from the ratio of X chromosomes to sets of autosomes (X/A) before enhancement of activity can occur. While Maroni and Lucchesi (1980) have shown that this deviation can be fractional, in nature it usually consists of the absence of an entire X chromosome in males. It is therefore reasonable to contend that such programmed regulation of transcription must involve the interaction of *trans*-acting factors with X-linked *cis*-acting sequences. The observation that cloned autosomal genes relocated to the X chromosome are often compensated suggests that the X-linked *cis*-acting regulatory sequences responsible for enhancement of gene activity in males are widely distributed.

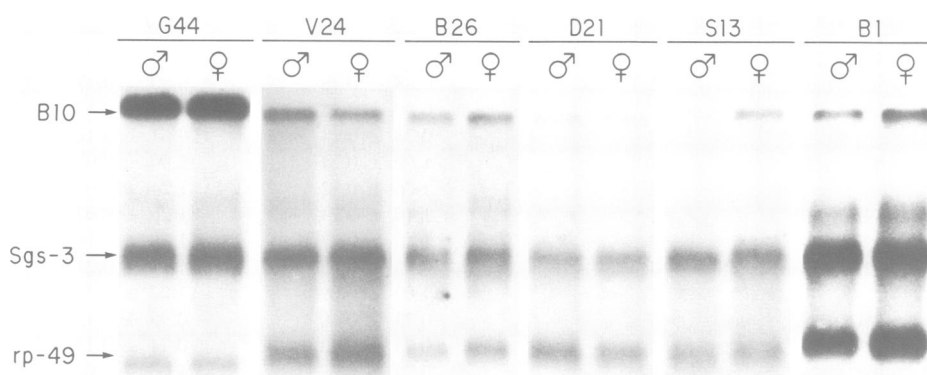
The *LSP1- $\alpha$*  gene of *D. melanogaster*, although normally present on the X chromosome, is not dosage compensated. The primary purpose of the experiments reported in this paper was to determine if this gene is inherently incapable of responding to the compensatory mechanism. Were this not the case, one may reasonably conclude that *LSP1- $\alpha$*  is not compensated because it happens to reside outside of

**Table III.** Dosage effects on steady-state levels of *LSP1- $\alpha$ B10* transcripts in females

Line	Heterozygous/homozygous females <sup>a</sup>	n <sup>b</sup>
F53	0.64 $\pm$ 0.08	4
G44	0.51 $\pm$ 0.07	3
B26	0.58 $\pm$ 0.18	2

<sup>a</sup>Mean male-to-female ratios of the counts hybridized to *LSP1- $\alpha$ B10* RNA relative to *Sgs-3* RNA and their standard errors. Hybridization probes are as described in Table I.

<sup>b</sup>Number of independent RNA extractions.



**Fig. 5.** Examples of the Northern blots used to quantify the relative steady-state level of *LSP1- $\alpha$ B10* A + RNA in transformant lines. Amounts of A + RNA electrophoresed were 1.5–2.0  $\mu$ g/lane. Hybridization and quantitation of radioactivity was as described in Figure 4 and Table I. The results are shown in Table II.

the influence of the regulatory sequences responsible for enhancing the transcription of X-linked genes in males. The data strongly favor this latter explanation.

Of some considerable interest is the fact that one of the *LSP1- $\alpha$ B10* transformants (line G44), as well as several autosomal genes which have been transposed to the X chromosome, show only partial dosage compensation. An explanation for this occurrence may be found in a general consideration of dosage compensation as it is exhibited by normal X-linked genes at their indigenous location. It is well known that there exist differences in the strength of the gene promoters, i.e. variations in their ability to initiate transcription. Given the great variety of genes present on the X chromosome, it is reasonable to assume that their promoters represent a spectrum of different strengths. Yet, dosage compensation leads almost invariably to a rather precise 2-fold increase in activity of X-linked genes in males. This uniform level of enhancement may require a differential set of interactions between promoters and compensatory sequences. These sequences may be similar to traditional enhancers, or they may consist of specific short sequences (such as the one proposed by Jaffe and Laird, 1986) or of poly(dC–dA/dG–dT) stretches (proposed by Huijser *et al.*, 1987; Pardue *et al.*, 1987). Regardless of their nature, the number of compensatory sequences and their position in relation to a given X-linked gene must be such that a 2-fold enhancement of that gene is achieved in males. In order to achieve this precise level of compensation, it is likely that considerable differences may be found in the number and spatial relationship of compensatory sequences and the genes which they influence. It also follows that the distribution of these sequences along the X chromosome would be non-random. Given such a non-random distribution of regulatory sequences, a cloned non-dosage compensated X-linked or autosomal gene relocated to the X chromosome may fail to become compensated or may exhibit a wide range of hyperactivation in males. For example, transposition of a gene to a position on the X chromosome that fortuitously places it under the influence of a distant compensatory sequence may result in 'undercompensation' of the gene in males. It might be expected, therefore, that excessive, normal or partial compensation, as in the case of line G44, will depend on the nature of the gene's promoter and its interaction with the compensatory sequence(s) present in the ectopic chromosomal domain.

## Materials and methods

### Tagging of the *LSP1- $\alpha$* gene and construction of the transformation vector

The *LSP1- $\alpha$*  gene, contained in a 9.4 kb *EcoRI* genomic fragment cloned in pBR322 (Smith *et al.*, 1981), was the generous gift of Dr David Glover. B10 is a 500 bp *BamHI* fragment of coding sequence from the mouse L1 repeat (Martin *et al.*, 1984) kindly provided by Dr Sandy Martin. The *LSP1- $\alpha$*  clone was partially digested with *BamHI* to produce linear molecules and the linear form of the clone was isolated from a 0.8% agarose gel. Ligations were performed with T4 ligase using the linearized *LSP1- $\alpha$*  clone treated with calf intestinal phosphatase and purified B10 fragment. Ligation products were used to transform HB101 cells which were plated on ampicillin plates (Maniatis *et al.*, 1982).

Approximately 100 colonies were transferred and tested (Grunstein and Hogness, 1975) for hybridization with a B10 probe. Plasmid DNA was isolated from positive colonies and the location of the B10 insert was determined by restriction analysis in combination with Southern blots and hybridization to the B10 probe. This was necessitated by the presence of five *BamHI* sites in the *LSP1- $\alpha$*  sequence and one *BamHI* site in pBR322 into which the B10 fragment could have inserted. One clone carrying the

insert in one of the two 3'-most *BamHI* sites was selected and designated *LSP1- $\alpha$ B10*. A 9.9 kb *EcoRI* fragment containing this hybrid gene was isolated and inserted into the P-element transformation vector pPA-1 kindly provided by Dr J.W. Posakony. This vector contains the P-element sequences necessary for transposition and *Adh*<sup>+</sup>, the structural gene for alcohol dehydrogenase which provides a selectable marker for successful transformation. The pPA-1 plasmid vector was cut with *EcoRI*, treated with phosphatase, mixed with purified 9.9 kb fragment and ligated with T4 ligase (Maniatis *et al.*, 1982). Following transfection and plating of HB101 cells, colonies which hybridized to a B10 probe were selected. Plasmid DNA was isolated and subjected to restriction analysis to determine the orientation of the *LSP1- $\alpha$ B10* insertion. One clone was chosen and designated pELBA.

### *Drosophila* transformation and selection of transformants

Pre-blastoderm embryos of genotype *Adh*<sup>F<sup>6</sup></sup>, *cn/Adh*<sup>F<sup>6</sup></sup> *cn*; *ry*<sup>506</sup>/*ry*<sup>506</sup> were injected, following the procedure of Rubin and Spradling (1982, 1983) and Karess and Rubin (1984), with pELBA DNA (300 µg/ml) and helper plasmid *p $\pi$ 25.7wc* DNA (80 µg/ml). Injected embryos (G0) were reared to adulthood and mated to individuals from the same strain. Their adult progeny (G1) were tested for the presence of transformants by positive selection with ethanol by the procedure of Vigue and Sofer (1976) modified by Goldberg (1980). Homozygous lines were established by inbreeding and tested for homozygosity by outcrossing to the recipient strain.

### *In situ* hybridization to polytene chromosomes

A *HindIII* subfragment of the cloned *LSP1- $\alpha$*  gene was labeled with a biotinylated nucleotide by nick-translation and allowed to hybridize to its homologous sequences on larval salivary gland polytene chromosomes; its presence was detected by the binding of a streptavidin–biotin–horseradish peroxidase complex (ENZO Biochem Inc.) according to a method modified by E. Hafen (personal communication).

### RNA blot analysis

Late third instar larvae were classified according to sex using the size of the gonad anlagen as criterion. Total RNA was extracted from samples of 50 individuals as described in Levy and Manning (1982). RNA gels were prepared according to Thomas (1980) and Southern blots according to Southern (1975). Blots were hybridized with <sup>32</sup>P-labeled probes which consisted of the *HindIII* fragment of the cloned *LSP1- $\alpha$*  gene, or of a mixture of the B10 fragment, a 5.7 kb *EcoRI* fragment from  $\lambda$ CDM2008 containing the *Sgs-3* sequence (Meyerowitz and Hogness, 1982), and a 639 bp *EcoRI*–*HindIII* fragment from pGRP49 containing the *rp49* sequence (O'Connell and Rosbash, 1984). Following hybridization and autoradiography, appropriate regions of the filter were cut out, using the exposed film as a template, and their radioactivity counted. The ratio of counts hybridized to *LSP1- $\alpha$ B10* RNA to counts hybridized to *Sgs-3* and *rp49* RNA was calculated for each sample and used in the comparison of the relative level of expression of the transduced hybrid gene between males and females (Tables I and II).

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